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(57) Abstract

Peptides are disclosed that are capable of effective recognition of the human IgE Fcε receptor. The peptides partially comprises a fragment of human IgE. By addition of constraining amino acid residues at or near the polypeptides' N- and C-termini, these peptides may be forced to assume a loop configuration. These constrained peptides are useful as competitors of human IgE for the Fcε receptor, and may be used to block the development of type I hypersensitivity.

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INHIBITION OF IgE-MEDIATED ALLERGIES BY A HUMAN IgE-DERIVED OLIGOPEPTIDE

This application claims priority from U.S. provisional application serial No. 60/031,991, filed December 6, 1996.

BACKGROUND OF THE INVENTION

Human IgE is believed to provide immunity to parasites. It also mediates Type I hypersensitivity. Type I hypersensitivity is an allergic response leading to the manifestation of such symptoms as hay fever and asthma. Briefly, on encountering an antigen such as pollen, B-cells commence synthesis of antigen-specific IgE. The antigen-specific IgE then binds via its Fc region to cellular class specific receptors and, thereafter, any further encounter with the antigen triggers degranulation of the cells releasing mediators. This is the cause of the acute inflammatory symptoms, hay fever and asthma, typical of Type I hypersensitivity.

Like all immunoglobulin, IgE comprises two heavy and two light chains. The ε heavy chain consists of five domains, one variable domain VH, and four constant domains CH1 to CH4. The molecular weight of IgE is about 190,000 kd. The heavy chain is approximately 550 amino acid residues in length.

A 330 amino-acids long polypeptide, corresponding to amino acid residues 218 to 547 of the ε heavy chain of IgE was shown to have an inhibitory effect on the release of mediators from human mast cells. The ε chain polypeptides apparently form dimers, *i.e.*, two 330 amino acid long chains linked by disulfide bonds. See *Nature* **315**: 577-78 (1985). The numbering of amino acid residues in the chain corresponds to that proposed by Bennich for hIgE. See Bennich, *PROGRESS IN IMMUNOLOGY II*, **Vol. I**, at pages 49-58 (1974).

Strategies that block the initial sensitization of target cells with antigen specific IgE have been explored following the demonstration that human (h) myeloma IgE-derived Fcε fragments generated by papain cleavage, comprising hε-chain residues 227-547, can competitively inhibit the binding of IgE to cells expressing high-affinity receptor (FcεR1). See Stanworth *et al.*, *Lancet* 2: 17-

20 (1968); Ishizaka *et al.*, *Immunochemistry* **7**: 687-94 (1970). This observation initiated the quest for progressively smaller peptides as potential IgE antagonists.

More recent studies aimed at the identification of the receptor binding site(s) employed chimeric human/mouse IgE antibodies, ε/γ chimeras, site-specific mutagenesis, anti-IgE antibodies, or IgE derived peptides. See Robertson *et al.*, *Mol. Immuno.* **25**: 103-108 (1988); Stanworth, *et al.*, *Lancet* **336**: 1279-1283 (1990); Nio *et al.*, *Peptide Chemistry* **2**: 203-206 (1989); Weetal *et al.*, *J. Immuno.* **145**: 3849-3854 (1990); Nissim *et al.*, *EMBO J.* **10**: 101-107 (1991); Nissim *et al.*, *Mol. Immuno.* **29**: 1065-1072 (1992); Basu *et al.*, *J. Biol. Chem.* **268**: 13118-13127 (1993); Nissim *et al.*, *J. Immuno.* **150**: 1365-1374 (1993); and Presta *et al.*, *J. Biol. Chem.* **269**: 26368-26373 (1994). They indicate that the site(s) in IgE that interact(s) with Fc-receptors depend(s) on structures associated with residues located in the Cε3 domain, although Cε4 involvement has also been invoked. See especially Basu *et al.*, *supra*; Stanworth, *et al.*, *supra* (1988).

Extensive research to date has pointed to a number of apparently contradictory assignments regarding the mode of action and efficacy of such peptides. In part, this can be attributed to the assay employed for the assignation of biological activity. It has been claimed that short peptides containing between 5-76 amino acids, derived from a region spanning amino acids 265-547 of the Fcε region will block the binding of human IgE to class-specific high- and/or low-affinity Fc receptors. See Hamburger *Science* **189**: 389 (1975); Helm *et al.*, *Nature* **331**: 180 (1988); Helm *et al.*, *Proc. Natl. Acad. Sci.* **186**: 9465 (1989); Helm *et al.*, *Progress in Allergy and Clinical Immunology* **2**: 589 (1992); Weetal *et al.*, *J. Immuno.* **145**: 3849 (1990); Nissim *et al.*, *supra* (1991); Nissim *et al.*, *supra* (1992); Nissim *et al.*, *supra* (1993); *Basu et al.*, *supra* (1993); Stanworth *et al.*, *supra* (1990); Robertson *et al.*, *Mol. Immuno.* **25**: 103 (1988); Presta *et al*, *supra*; Nio *et al.*, *supra* (1989); Nio *et al.*, *FEBS Lett.* **319**: 225 (1993). Generally, Fcε-derived peptides tested for the inhibition of binding of native antigen specific IgE have a lower affinity for the receptor than the native ligand. See Helm *et al.*, *Progress in*

Allergy and Clinical Immunology 2: 589 (1992). They are therefore unlikely to act *in vivo* as effective competitive inhibitors.

SUMMARY OF THE INVENTION

An object of the present invention is to provide new oligopeptide(s) that are stable and highly active in anti-allergy treatment. Another object of the invention is to provide a method of preparing the oligopeptide by heterologous expression and isolation. Alternatively, the oligopeptide may be synthesized by known chemical synthetic methods.

Still another objective of the invention is to provide antibodies raised against the oligopeptide which can bind and thus inhibit IgE.

It is a further object of the invention to provide a pharmaceutical preparation in which the active ingredient is one of the oligopeptides. The preparation may also include a pharmaceutical carrier to permit clinical administration of the oligopeptide. It is yet another object of the invention to provide a pharmaceutical preparation in which the active ingredient is an antibody raised against one of the oligopeptides of the invention.

It is yet another object of the invention to provide a diagnostic method for estimating the level of IgE antibody in a sample. It is still a further object of the invention to provide a method for estimating the level of IgE antibody bound to Fcε receptors on FC_ε receptors expressing cells.

It is also an object of the present invention to facilitate targeting by drugs or diagnostic agents of cells that bear IgE high-affinity receptors.

In achieving these and other objects, there is provided, in accordance with one aspect of the invention, oligopeptides that interact with the Fcε receptor, wherein the oligopeptide comprises:

P S P F D L F I R K S (Seq. I.D. No. 1)

In accordance with another aspect of the invention, there has been provided oligopeptides that interact with the Fcε receptor,

wherein said oligopeptide has been constrained to form a loop structure. For example, the oligopeptide can include C L S R at the N-terminal and P T I T S C C at the C-terminal (see Seq. I.D. No. 2).

In accordance with another aspect of the invention, there has been provided pharmaceutical preparations comprising an oligopeptide that has been constrained to form a loop structure.

In accordance with a further aspect of the invention, there has been provided DNA molecules encoding the oligopeptide and biological systems that express the DNA molecule. In a preferred embodiment, the biological system is a prokaryotic or eukaryotic cell.

In accordance with still another aspect of the invention, there has been provided methods of preparing an oligopeptide competitor of IgE, comprising: culturing of the prokaryotic or eukaryotic cell and purifying the oligopeptide.

In accordance with yet another aspect of the invention above, there has been provided competitive binding assays for hIgE wherein the competitor is the oligopeptide competitor of IgE.

In accordance with still another aspect of the invention, there has been provided antibodies raised against the oligopeptide competitor of IgE.

In accordance with yet a further aspect of the invention, there has been provided pharmaceutical preparations comprising antibodies raised against the oligopeptide competitor of IgE.

In accordance with still a further aspect of the invention, there has been provided diagnostic methods for determining IgE levels in a sample of fluids comprising the steps of:

(a) contacting said sample with an antibody raised against an oligopeptide competitor to IgE; and

(b) precipitating IgE with said antibody.

In accordance with yet another aspect of the invention, there has been provided diagnostic methods for determining available Fcε receptors on the membrane of cells, comprising the steps:

(a) providing a sample of such cells;

(b) providing a labeled oligopeptide competitor to IgE; and

(c) determining the amount of label bound by said cells.

In accordance with still another aspect of the invention, there has been provided methods for targeting a cell which expresses FcεR1 receptor, comprising delivering an oligopeptide according to the present invention to an organism.

Kits for performing the above methods also are provided.

Proteins that interact with the FcεR1 receptor, wherein the proteins comprise the amino acid sequence P S P F D L F I R K S (Seq. I.D. No. 1), are provided. Also provided are proteins that compete with IgE for the FcεR1 receptor, wherein the proteins comprise C L S R P S P F D L F I R K S P T I T S C C (Seq. I.D. No. 2). Proteins that compete with IgE for the FcεR1 receptor, wherein the protein consists essentially of (i) an amino acid sequence P S P F D L F I R K S, and (ii) constraining residues that flank the amino acid sequence of (i), wherein the constraining residues include cysteine and are within 16 residues N-terminal and 4 residues C-terminal of the amino acid sequence of (i), also are provided.

These and other aspects of the invention will become apparent to the skilled person in view of the disclosure and data contained herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. **Gene constructs, expression products, and mapping of receptor binding regions in human IgE.** Recombinant ε-chain gene fragments were subcloned into the multiple cloning site (*MCS*) of the bacterial expression plasmids pGEX-3X and pGEX-KG and expressed in *E. coli*. See Ho *et al.*, *Gene (Amst.)*, **52**: 2940-2948 (1989), Helm *et al.*, *supra* (1988), and Helm *et al.*, *Eur. J. Immuno.* **21**: 1543-1548 (1991). The ε-chain expression plasmids pSV-V_{NP}hε/rε were employed for the construction of mutant and chimeric IgE molecules and expressed in the J558L myeloma cell line. See Helm *et al.*, *supra* (1991). *Panels A* and *B* summarize the ability of the truncated, chimeric, and mutant ε-chain variants to bind to FcεRIα expressed on RBL-2H3.1 and RBL-2/2/C cells and to FcεRII expressed on the 8866 lymphoblastiod cell line. See Wilson *et al.*, *Eur. J. Immuno.* **23**: 240-244 (1993) and Helm *et al.*, *supra* (1991). Initial screening for

biological activity was determined by assessing the capacity of GST·ε-chain fusion proteins to inhibit the binding of ^{125}I-labeled hIgE (1 nM) to the receptors. For a description of GST·ε-chain fusion proteins see Helm *et al.*, *J. Biol. Chem.* **271**: 7494-7500 (1996). The degree of inhibition effected by nonbinding ligands was identical, within limits of experimental error, to that observed with GST, which was included as a negative control (see Figure 2). Purification of truncated recombinant GST·ε-chain fusion proteins and mutant and chimeric IgE molecules was carried out as described in Examples. Ligands were labeled with ^{125}I for direct binding studies (see Table I). Nonbinding ligands showed no binding above background levels even at concentrations above 10^{-5} M. *Panels C* and *D* show GST·ε-chain fusion proteins that were immuno precipitated with a rabbit anti-GST serum, followed by SDS-PAGE (12%) separation under nonreducing conditions and immunoblotting with a horseradish peroxidase-labeled rabbit anti-human IgE serum. *Panel C, lanes 1-6*, GST·ε-(226-547), GST·ε-(226-361), GST·ε-(226-357), GST·ε-(226-354), GST·ε-226-352), GST·ε-(226-340), *Panel D, lanes 1-6*, GST·ε-(326-547), GST·ε-(340-547), GST·ε-(343-547), GST·ε-(345-547), GST·ε-(350-547), GST·ε-(226-440)-Cγ3. +, receptor binding; -, loss of receptor binding; *n.d.*, not determined.

Figure 2. **Percentage of inhibition of ^{125}I-hIgE binding to RBL-2/2/C cells by native and recombinant hIgE-derived ε-chain fragments.** To measure the inhibition (IC_{50}) of 1^{25}I-hIgE to RBL-2/2/C cells by native IgE and recombinant ε-chain fragments, cells were preincubated at 22°C for 1 h with increasing concentrations (10_{-12} - 10_{-5} M) of each of the unlabeled GST fusion peptides in 125 μl of binding buffer or, as a negative control, GST or binding buffer. ^{125}I-hIgE was then added (1 nM). After 45 min. the cells were washed twice with 0.5 ml of binding buffer and lysed in the same volume of lysis buffer, and aliquots were removed for γ-counting. The IC_{50} values for GST·ε-(226-547) and GST·ε-(226-357) (data not known) were identical to those observed for hIgE (▲) and GST·ε-(226-354) (△). In GST·ε-(226-340) and GST·ε-(335-547), the sequence common to all fragments that can engage FcεRI has been deleted by either the N- or C-terminal truncation. GST·ε-(226-340) (◇) and GST·ε-(335-547) (■)

show inhibition levels similar to that obtained with GST (⊡) and all other fragments classified as nonbinding ligands in Figure 1. ♦, GST·ε-(340-547); ○, GST·ε-(440-547). Data shown represent the means of at least three separate experiments carried out in duplicate.

Figure 3. **Drawing of the α-carbon trace of a model structure for hIgE with various fragments and domains indicated.** The light chains are drawn with thin lines and the heavy chains with thick lines, one thicker than the other. The interchain Cys at position 328 is labeled. The 11-amino acid segment 343-353, which is common to all IgE-derived peptides that bind to FcεRI, is drawn with larger circles and wide, empty bonds in heavy chains.

Figure 4. **pH profile for the binding of native IgE and recombinant IgE-derived peptides to RBL-2/2/C cells.** hα-chain-transfected RBL-2/2/C clones were distributed into 48-well plates at 10^5 cells/well and incubated with 10^{-6} M dexamethasone for 24 h at 37°C. Prior to the assay cells were washed twice with 0.5 ml of saline containing 0.4% BSA and preincubated with 125 μl of 50 mM phosphate-buffered saline containing 0.4% BSA (pH range 5.9-8.1) for 10 min at 37°C before adding 50 μl of 2 μg of ^{125}I-labeled IgE (⊡), 0.7 μg of GST·ε-(340-547)(♦), and GST·ε-(226-354)(⊡). Cells were incubated for 30 min, after which unbound ligand was removed by washing with binding buffer before measurement of cell-bound label. Results were corrected for nonspecific binding. (Data shown represent the means of two determinations carried out in duplicate.)

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

1. *Definitions*

Core Sequence: In the present invention, "core sequence" refers to an 11 amino acid sequence which has been identified as the minimal region of IgE required for interaction with Fcε receptors.

Constrained: In the present invention, a "constrained" molecule is a short molecule, typically an oligopeptide, comprising residues that affect the shape of the oligopeptide. The constraint is introduced by residues near or at the ends of the oligopeptide. Such residues link to each other, generally by forming disulfide bonds which constrain the shape of the molecule into a loop-like

structure. Often the residues involved in creating a constraint are cysteines.

Loop Configuration: Model studies have suggested that the IgE region that interacts with the Fcε receptor forms a loop-like structure. See Helm *et al.*, *supra* (1991) and Padlan & Helm, *Biochem. Soc. Transactions* 21: 963-967 (1993). According to this description, "loop" refers to a native-like configuration achieved by an oligopeptide comprising the core sequence and further constrained as above, so that the ends of the oligopeptide are in close proximity.

Residue: The IgE competitor molecule of the invention includes oligopeptides. Its constituents or chemical entities (its subunits) of the competitor molecule typically are amino acids. According to some embodiments, however, other entities can be introduced into what is still called an "oligopeptide" in this description. Those may be modified amino acids or other groups possessing desired features. For example, any chemical residue that has a carboxyl group may allow formation of a covalent bond to another molecule. Also, some non amino acid residues, such as methylene, carboxymethylene, and carboxidiimide, may allow disulfide-like bonds within the oligopeptide.

Oligopeptide: The oligopeptide of the present invention is defined as a molecule of about 50 residues or smaller. Part of the sequence of this molecule is the core sequence defined above. The oligopeptide is constrained as defined above, by additional residues.

2. Overview

It has been discovered for the first time that a particular peptide, Pro343-Ser353, is common to all polypeptides that can bind the high- and low-affinity IgE receptors. An understanding of the native structure of the binding domain by three-dimensional modelling of the binding site for IgE was developed. These studies have provided information regarding the structural motif that is required for an oligopeptide to be effective in mimicking IgE in its interaction with class-specific Fc receptors. Based on those findings, model constrained oligopeptides were proposed and tested for binding Fcε receptors. The testing of those oligopeptides

includes a determination of the rate of association and disassociation to receptors, and their ability to compete with IgE. Methods for testing such activity have been described. See Wilson *et al., supra;* Wilson *et al., J. Clin. Immunoassay* **16**: 91-95 (1993); and Helm *et al., supra* (1991).

A useful oligopeptide would be relatively small, so that it will be easy to synthesize and deliver. An additional advantage of a small but effective oligopeptide is that its therapeutic/ prophylactic use is less likely to trigger an adverse, *i.e.* anaphylactic, immune response.

However, a small peptide might not assume the structure of the segment as found in the intact molecule. The three-dimensional structure of a polypeptide is determined by its amino-acid sequence and its environment and conformation. Fragments which are too small will not have the environment found in the intact molecule. Such fragments, freed from structural constraints, will assume many other conformations. See Wright *et al., Biochem.* **27**: 7167 (1988). The probability that a peptide will assume the proper conformation, *i.e.* that which is found in the intact molecule is very small. Improper configuration will likely be manifested in a greatly reduced binding affinity. Low binding affinities will require the administration of large doses of peptide for effectiveness. This difficulty can be obviated by imposing structural constraints on the peptide segment.

According to one embodiment of the invention, a molecule was designed that comprises the core sequence that is required for the binding of IgE to its receptors. This embodiment is further constrained so that its structure mimics that which has been predicted for the segment as found in the intact IgE molecule. The sequence is:

C L S R P S P F D L F I R K S P T I T S C C (Seq. I.D. No. 2).

The oligopeptide of the embodiment thus comprises an IgE derived sequence extending past the core sequence: L S R P S P F D L F I R K S P T I T which is the Leu340-Thr357 fragment from the human IgE sequence, and additional amino acids at the N- and C- termini of the peptide. In those sequences, the Pro343-Ser353 core fragment P S P F D L F I R K S (Seq. I.D. No. 1) is underlined.

In the one embodiment, the Ser after Thr357 was added to improve the solubility of the peptide; other amino acids, such as Thr, Asp, Asn, Glu, Gln, Tyr, or His could have been used also. The starting and penultimate Cys were added to provide the intramolecular disulfide linkage. Thus the oligopeptide forms a loop and is active only when incubated under conditions that promote disulfide bond formation. The C-terminal cysteine was added for possible linkage to a carrier molecule or pharmacologically active agent; such linkage may not be needed or desired, depending on the chosen application or mode of administration of the oligopeptide. Other amino acids could be used to link to a second molecule and may be introduced at either the N- or the C-terminus.

In other embodiments, longer or shorter oligopeptides which contain the residues required for binding to receptor, likewise constrained by disulfide bonds or the like, also will be effective. Examples of shorter, constrained oligopeptides are:

C S R P S P F D L F I R K S P T I T C C (Seq. I.D. No. 3)

C R P S P F D L F I R K S P T S C C (Seq. I.D. No. 4)

C P S P F D L F I R K S P T C C (Seq. I.D. No. 5)

where the underlined sequence is the core sequence required for binding the receptor.

According to other embodiments, the terminal Cys may or may not be needed or desired, depending on whether the oligopeptide is to be linked to an additional effector molecule or not. In yet other embodiments, other amino acids may be substituted for residues outside the recognition motif, or within the recognition motif, to improve the solubility or other physical chemical property of the oligopeptide or to enhance its biological activity. Very small insertions or deletions (usually no more than a few amino acids) are also possible. Shorter and longer polypeptides may allow folding such that the core sequence is in a configuration like the native configuration. However, practically speaking, peptides outside certain lengths are less likely to achieve the desired configuration. In a preferred embodiment, the oligopeptide is fewer than 50 and more preferably 13-27 residues in length. Furthermore, other means of

constraining the recognition peptide to its loop-like conformation as found in the intact molecule may be used also.

Other chemical structures could be utilized to constrain the oligopeptide. For example, linkages involving methylene, carboxymethylene, carboxidiimide fragments, etc. have been utilized. See for example Dutta *et al, J. Med. Chem.* **33**: 2552-2560, 2560-2568 (1990); Sham *at al, J. Med. Chem.* **31**: 284-295 (1988); Thaisrivongs *et al., J. Med. Chem.* **31**: 13691376 (1988), and **34**: 1276-1282 (1991); Weber *et al., J. Med. Chem.* **34**: 2692-2701 (1991). It may also be desirable to replace groups inside or outside the receptor motif with peptidomimetic or other moieties for the purpose of improving the binding properties of the molecule, or its physical chemical properties. Such changes would result in an oligopeptide that adheres to the principles of the present invention and thus are within the scope of the invention.

Exemplary uses for an oligopeptide that mimics the epsilon (ε) heavy-chain region of IgE include the capacity (a) to inhibit the binding to class-specific receptors and thus prevent the release of cellular mediators from cells expressing the receptors, (b) to stimulate the formation of non-anaphylactic anti-IgE antibodies which can bind to membrane IgE and inhibit B-cell synthesis of IgE by down regulation of its synthesis, (c) when linked to a pharmacologically active agent, to selectively destroy cells expressing Fcε receptor-bearing cells, and (d) to inhibit IgE/CD23 facilitated antigen presentation. The oligopeptide thus can be used prophylactically or as a treatment. In addition, the constrained oligopeptide can be used *in vitro* to determine receptor availability and receptor occupancy by IgE. In such tests, the polypeptide, or IgE, can be labeled to enhance detection.

An antibody raised against such an oligopeptide also has utility due to its interception of IgE in the plasma, away from the Fcε receptor. Antibodies raised against the oligopeptide, as opposed to an anti-Ab antibody, are likely to display less cross-reactivity. Furthermore, antibodies raised against antibodies can sometimes mimic the original antigen and worsen the hypersensitivity state. Antibodies raised against the oligopeptide of the invention reduce

the risk of increasing hypersensitivity, since they do not recognize receptor bound IgE. The antibody can inhibit IgE/CD23 facilitated antigen presentation, and, when linked to a pharmacologically active agent, can selectively destroy cells expressing membrane IgE.

According to one aspect of the invention, antiserum is raised against any of the oligopeptides of the invention. According to one embodiment, polyclonal or monoclonal antibodies, including humanized antibodies are prepared. Such antibodies are used as a prophylactic or therapeutic treatment, by virtue of their binding to IgE in the plasma but not to IgE bound to cellular receptor.

According to one embodiment of the invention, sera was raised against the oligopeptide: C L S R P S P F D L F I R K S P T I T S C C (Seq. I.D. No. 2). It was shown that only oligopeptide pre-incubated under conditions that allow S-S bridges to form produced sera which can bind IgE in solution. Furthermore, the sera did not produce hypersensitivity. The antibody appears not to bind IgE when IgE is bound to the Fcε receptor.

It is well known that IgE plays a role in the response of an organism to parasites. See Hagan *et al.*, *Nature* **349**: 243-45 (1991). Furthermore, high levels of IgE have been correlated with AIDS. See Wright *et al.*, *J. Allergy Clin. Immun.* Feb: 445-52 (1990), and Lin *et al.*, *Ann. Allergy* **61**: 269-72 (1988). Thus, determination of either the IgE titer in a sample, or the level of expression or availability of Fcε receptor on cell surface would be desirable. Hence, labeled antibody raised against the constrained oligopeptide of the invention and the constrained oligopeptide itself, have useful application in diagnostic assays. According to one embodiment of the invention, the oligopeptide can be used to determine the level of expression of IgE receptors and/or the levels of receptor available to bind IgE. According to another embodiment, the titer of IgE in a sample can be estimated by employing antibody specific for the constrained oligopolypeptide.

3. A DNA Molecule Encoding the Oligopeptide of the Invention

According to one object of the invention, an oligopeptide is provided which mimics IgE in its ability to bind Fcε receptor.

According to one embodiment, the oligopeptide comprises Cys residues near the termini which constrain and thus shape a polypeptide into the desired configuration by providing intramolecular S-S bonding, *i.e.* forming a loop. According to a preferred embodiment, the oligopeptide further comprises additional amino acids which either do not affect its activity, or impart benefits, such as improved solubility, stability or an optimum size. According to a further preferred embodiment, a C-terminal or N-terminal amino acid is added to allow for covalent linkage of the oligopeptide to another molecule. The oligopeptide thus designed could be chemically synthesized. Chemical synthesis of peptides is well known to an artisan skilled in the field. It could allow the introduction of modified amino acids, or linkage of groups other than an amino acids.

However, in a preferred embodiment, the oligopeptide according to any of the embodiments above is a translation product of a gene encoding the oligopeptide. The gene, and the variations of the gene according to the embodiments above, can be produced by many methods. For example, a set of complementary oligonucleotides could be designed. If need be, "batch ligation" of sets of such oligonucleotides could be employed. That is, if the coding region is too long to be covered by one set of complementary oligonucleotides, ligation of sets of complementary oligonucleotides can be employed. In either case, the oligonucleotides would be so designed as to allow subsequent subcloning into an expression vector.

Alternatively, one can take advantage of the fact that this segment has been cloned and expressed as a GST fusion peptide which does not form a loop. See Helm *et al.*, *supra* (1996). PCR or NASBA could be applied, employing mutagenic primers, so a subsection of the DNA containing changes would be amplified and subcloned. Yet another alternative would be that a section of interest from the gene would be subcloned (by amplification as above, or by the "cut and paste" of molecular cloning) into a vector that can produce single stranded DNA. This would facilitate employment of mutagenic primers, employing well known techniques and commercially available kits. Finally, combinations of the above methods, to allow further

variation of a gene sequence encoding the desired oligopeptide, could be employed by one skilled in the art. The changes envisioned, at the protein level, could result in substitution, insertion or deletion of a few amino acids.

Substitutional variants typically contain the exchange of one amino acid for another at one or more sites within the protein, and are designed to modulate one or more properties of the protein such as stability against proteolytic cleavage. Substitutions could be conservative, that is, one amino acid is replaced with one of similar shape and charge. Conservative substitutions are well known in the art and include, for example, the changes of: alanine to valine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine, glutamine, or glutamate; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine. Changes involving less conservative substitutions also can be made according to the invention.

Insertional variants may alter the sequence of the oligopeptide competitor of IgE or may be used to create fusion proteins such as those used to allow rapid purification of the protein (see below). Insertional variants may also produce hybrid proteins containing sequences from other proteins. Other insertional variants can include those in which additional amino acids are introduced within the coding sequence of the protein. These typically are smaller insertions than the fusion proteins described above and are introduced, for example, to disrupt a protease cleavage site or modify chemical or biological properties of the oligopeptide. Deletional variants would remove one or a few amino acids from the oligopeptide.

In one embodiment, peptides produced from translation of a gene are subjected to further chemical manipulation, so modification of the peptide is achieved.

The methods employed to introduce such changes are well known in the art. Some often-used manuals describing relevant experimental protocols and techniques are Ausubel, ed., in CURRENT PROTOC. MOLEC. BIOL., vols. 1-3, Wiley Interscience Publ., and also Murray ed., METH IN MOLEC. BIOL., especially vol. 7, GENE TRANSFER AND EXPRESSION PROTOC., (1991); vol. 9, PROTOC. IN HUMAN MOL. GENE., Mathew ed., (1991); and vol. 15, PCR PROTOC., White ed., (1993), Humana Press, Totowa, NJ. See also U.S. Patent No 5,409,818.

4. ***Expression and Purification of the oligopeptide***

According to another embodiment of the invention, a DNA encoding an oligopeptide sequence as described above is used to recombinantly produce protein. For this purpose, the DNA is operably linked into a vector to transcriptional/translational features, according to methods well understood by those skilled in the art. Typically, the DNA sequence encoding a protein/peptide product will be inserted downstream from a promoter. Downstream of the coding sequence there will be a terminator sequence. In general, host-cell specific sequences improving the production yield will be used and appropriate control sequences will be added to the expression vector, such as ribosome binding sites, and, in eukaryotes, enhancer sequences, and polyadenylation sequences. The construct can be expressed in a variety of systems; for example in mammalian cells, baculoviruses, bacteria and yeast, as discussed below.

Each such host system would have advantages and disadvantages to be considered in any given circumstance. For example, bacterial expression would be easy, inexpensive and efficient. Proper folding or modifications, or potentially of more immediate interest in the present invention, disulfide bond formation *in vivo*, would necessitate use of eukaryotic cell systems. Those choices are rationally made by the skilled artisan in light of the specific gene fragment to be expressed, and its ultimate use. Vectors for expression in each such host are commercially available.

Thus, the present invention also relates to a host cell stably transformed with any of the above described DNAs of the invention, wherein the transforming DNA is capable of being expressed. Such

host cell can be a prokaryote or eukaryote. Preferred host cells include bacterial, insect, mammalian and yeast cells, or whole organisms such as insects or plants. Methods for the transformation of such host cells with the DNA of the present invention and methods of culturing the same would be well-known to the skilled artisan.

In a preferred embodiment, oligopeptides according to the invention are cloned and expressed in *Escherichia coli (E. coli)*. Larger e chain fragments are known to express in *E. coli*. See *Eur. J. Immuno.* **15**: 966-69 (1985); and *Proc. Nat'l Acad. Sci. USA* **81**: 2955-59 (1984). Furthermore, small peptides have been expressed as GST fusion peptides which could be cleaved from GST by thrombin. See Helm *et al., supra* (1996).

5. Antibody Production and Related Methods

Antibodies against the oligopeptide of the invention can be obtained using the product of an expression vector as an antigen. The preparation of polyclonal antibodies is well-known to those of skill in the art. See, for example, Green, "*Production of Polyclonal Antisera*," in *IMMUNOCHEMICAL PROTOCOLS*, Manson, ed., pages 1-5, *Humana Press* (1992). In a preferred embodiment, rabbit polyclonal sera was prepared against one of the oligopeptides of the invention.

Alternatively, an anti-oligopeptide antibody of the present invention may be derived from a rodent monoclonal antibody (MAb). Rodent monoclonal antibodies to specific antigens may be obtained by methods known to those skilled in the art. *See*, for example, Kohler and Milstein, *Nature* **256**: 495 (1975), and Coligan (eds.), *CURRENT PROTOCOLS IN IMMUNOLOGY*, VOL. 1, pages 2.5.1-2.6.7 (John Wiley & Sons 1991). Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising an antigen, verifying the presence of antibody production by removing a serum sample, removing the spleen to obtain B-lymphocytes, fusing the B-lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones which produce antibodies to the antigen, culturing the clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures.

MAbs can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography. See, for example, Coligan at pages 2.7.1-2.7.12 and pages 2.9.1-2.9.3. Also, see Baines *et al.*, "*Purification of Immunoglobulin G (IgG),*" in *METHODS IN MOLECULAR BIOLOGY,* **VOL. 10**, pages 79-104 (*The Humana Press, Inc.* 1992).

An anti-polypeptide antibody of the present invention may also be derived from a primate. For example, general techniques for raising therapeutically useful antibodies in baboons may be found, for example, in Goldenberg, international patent publication No. WO 91/11465 (1991), and in Losman *et al.*, *Int. J. Cancer* **46**: 310 (1990).

Alternatively, a therapeutically useful anti-oligopeptide antibody of the present invention may be derived from a "humanized" monoclonal antibody. Humanized monoclonal antibodies are produced by transferring mouse complementarity determining regions from heavy and light variable chains of the mouse immunoglobulin into a human variable domain, and then, substituting human residues in the framework regions of the murine counterparts. The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with the immunogenicity of murine constant regions. General techniques for cloning murine immunoglobulin variable domains are described, for example, by the publication of Orlandi *et al.*, *Proc. Nat'l Acad. Sci. USA* **86**: 3833 (1989), which is incorporated by reference in its entirety. Techniques for producing humanized MAbs are described, for example, by Jones et al., *Nature* **321**: 522 (1986), Riechmann *et al.*, *Nature* **332**: 323 (1988), Verhoeyen *et al.*, *Science* **239**: 1534 (1988), Carter *et al.*, *Proc. Nat'l Acad. Sci. USA 89*: 4285 (1992), Sandhu, *Crit. Rev. Biotech.* **12**: 437 (1992), and Singer *et al.*, *J. Immuno.* **150**: 2844 (1993).

As an alternative, an antibody of the present invention may be derived from human antibody fragments isolated from a combinatorial immunoglobulin library. See, for example, Barbas *et al.*, *METHODS: A Companion to Methods in Enzymology* **2**: 119 (1991), and Winter *et al.*,

Ann. Rev. Immuno. 12: 433 (1994), which are incorporated by reference. Cloning and expression vectors that are useful for producing a human immunoglobulin phage library can be obtained, for example, from STRATAGENE Cloning Systems (La Jolla, CA).

In addition, antibody of the present invention may be derived from a human monoclonal antibody. Such antibodies are obtained from transgenic mice that have been "engineered" to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human heavy and light chain locus are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy chain and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described by Green *et al.*, *Nature Genet.* 7: 13 (1994), Lonberg *et al.*, *Nature* 368: 856 (1994), and Taylor *et al.*, *Int. Immuno.* 6: 579 (1994).

6. The oligopeptide as a Targeting Molecule

The oligopeptide of the invention, when covalently bound or simply associated to another agent, could, by virtue of its affinity for the FcεR1, deliver such other agent to a cell that expresses the receptor. In the examples given herein, a Cys at the C-terminus of the oligopeptide is designated to facilitate just such association. Such agent could include for example a drug, a radiolabeled molecule, or any agent that could be diagnostic, or therapeutic, or used as a research tool. For example, liposomes are one method of delivery of agents to cells in an organism. They are sometimes targeted to the cell type or tissue of interest by the addition to the liposome preparation of a ligand, usually a polypeptide, for which a corresponding cellular receptor has been identified. Examples of cell receptors previously targeted include the folate receptor, which has recently been identified as a prominent tumor marker, especially in ovarian carcinomas. KB cells are known to vastly overexpress the folate receptor. See Campbell *et al.*, *Cancer Res.* 51: 6125-32 (1991). Yet other targeting ligands have been examined for liposome

targeting including transferrin, protein A, ApoE, P-glycoprotein, α_2-macroglobin, insulin, asiolofetuin, asialoorosomucoid, monoclonal antibodies with a variety of tissue specificities, biotin, galactose- or lactose- containing haptens (monovalent and tri-antennary), mannose, dinitrophenol, and vitamin B12. The ligands are covalently conjugated to a lipid anchor in either pre-formed liposomes or are incorporated during liposome preparation. See Lee & Low, *J. Biol. Chem.* **269**: 3198-04 (1994); *Biochim. Biophys. Acta* **1233**: 134-44 (1995).

In one embodiment of the invention, the association of the oligopeptide of the invention with a delivery agent as described above includes association with additional targeting agents. For example, in order to gain access to the cytoplasm, the DNA molecule must overcome the plasma membrane barrier. In nature, viral fusion peptides facilitate the delivery of viral DNA into the cytoplasm by promoting viral membrane fusion with the plasma membrane. For recent reviews on this subject, see Stegmann *et al.*, *Ann. Rev. Biophys. Chem.* **18**: 187-221 (1989). For the influenza virus, the hemagglutinin (trimer) HA peptide N-terminal segment (a hydrophobic helical sequence) is exposed due to a conformational change induced by acidic pH in the endosome (pH 5-6), inserts into the target membrane, and mediates the fusion between the virus and the target endosomal membrane. See Weber *et al.*, *J. Biol. Chem.* **269**: 18353-58 (1994). Recently, several amphipathic helix-forming oligopeptides have been designed to imitate the behavior of the viral fusion peptide. See, for example, Haensler & Szoka, *Biocon. Chem.* **4**: 372-79 (1993).

Nuclear localization signal peptides, when attached covalently to a macromolecule such as a protein, have been shown to facilitate their translocation into the nucleus. See Goldfarb *et al.*, *Nature* **322**: 641-44 (1986); Shreiber *et al.*, *Med. Sci.* **8**: 134-39 (1992). By the combination of cellular targeting by the oligopeptide of the invention and nucleus targeting, yet other agents could be delivered in the nucleus of specific cells, for example DNA molecules.

7. Delivery of the Therapeutic Agents to a Patient

Delivery of any of the above therapeutic agents or antibodies requires administration to the patient of therapeutically effective doses. "Administering" the pharmaceutical composition of the present invention may be accomplished by any means known to the skilled artisan.

The pharmaceutical compositions are preferably prepared and administered in dose units. Solid dose units are tablets, capsules and suppositories. For treatment of a patient, depending on activity of the compound, manner of administration, nature and severity of the disorder, age and body weight of the patient, different daily doses are necessary. Under certain circumstances, however, higher or lower daily doses may be appropriate. The administration of the daily dose can be carried out both by single administration in the form of an individual dose unit or else several smaller dose units and also by multiple administration of subdivided doses at specific intervals.

The pharmaceutical compositions according to the invention are preferably administered intravenously. However, other routes of administration can be readily ascertained. Thus, the pharmaceutical compositions can be administered topically, intravenously, orally or parenterally or as implants, even rectal use as appropriate. Suitable solid or liquid pharmaceutical preparation forms are, for example, granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, creams, aerosols, drops or injectable solution in ampule form and also preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are customarily used as described above. The pharmaceutical compositions are suitable for use in a variety of drug delivery systems. For a brief review of present methods for drug delivery, see Langer, *Science*, 249: 1527-1533 (1990), which is incorporated herein by reference.

The pharmaceutical compositions according to the invention may be administered locally or systemically. By "therapeutically

effective dose" is meant the quantity of a compound according to the invention necessary to prevent, to cure or at least partially arrest the symptoms of the disease and its complications. Amounts effective for this use will, of course, depend on the severity of the disease and the weight and general state of the patient. Typically, dosages effective *in vitro* may provide useful guidance in the amounts useful for *in situ* administration of the pharmaceutical composition, and animal models may be used to determine effective dosages for treatment of particular disorders. Various considerations are described, *e.g.*, in Gilman *et al.* (eds.) (1990) GOODMAN AND GILMAN'S: THE PHARMACOLOGICAL BASES OF THERAPEUTICS, 8th ed., Pergamon Press; and REMINGTON'S PHARMACEUTICAL SCIENCES, 17th ed. (1990), Mack Publishing Co., Easton, Pa.

The present invention, thus generally described, will be understood more readily by reference to the following examples, which are provided by way of illustration and are not intended to limit the present invention.

Example 1. *Gene constructs and site specific mutagenesis*

The numbering scheme for he--chain amino acid residues used in previous publications has been maintained. See Helm *et al.*, *supra* (1988); Vercelli *et al.*, *Nature* 338: 649-652 (1989); Helm *et al.*, *supra* (1989); Helm *et al.*, *supra* (1991). Herein, "h" stands for human and "r" stands for recombinant. The polymerase chain reaction (PCR) was used to amplify e-chain fragments comprising the entire Fc region (226-547), the Cγ3 from mouse IgG2a, the Cε2 domain (226-329), and the Ce4 domain (440-547). N-terminal deletions of the Fc region were prepared starting at amino acid residue positions 326, 330, 340, 342, 343, 344, 345, 350, and 355 and terminating at residue 547. C-terminal deletions were prepared starting at amino acid residue 226, and terminating at residues 361, 357, 354, 353, 352, 345 and 340. The DNA products were purified by agarose gel electrophoresis, digested with appropriate restriction enzymes, and subcloned into the bacterial expression plasmids pGEX-3X and PGEX-KG, which direct the synthesis of foreign polypeptides in *E. coli* as fusions with the 26 kDa glutathione-S-transferase (GST). See Guan *et al.*, *Anal. Biochem.*

192: 262-267 (1991). Cloning the recombinant ε-chain fragments in frame to the 3' end of the GST gene facilitates the production of large amounts of fusion protein (~500 mg/L). In addition to a versatile multiple cloning site, the vectors have been engineered so that the GST carrier can be cleaved off by digestion with coagulation factor Xa or thrombin. The initial screening for receptor blocking activity was carried out with partially purified GST-ε-chain fusion polypeptides. Following affinity purification on rabbit anti-GST affinity columns, and GST removal with thrombin, ε-chains showed identical receptor blocking capacities when compared to GST-ε-chain fusion polypeptides. Therefore this step was eliminated and all assays described in this study were carried out with affinity purified GST-ε-chain fusion polypeptides. Short GST fusion peptides comprising ε-chain residues 338-359 and 340-357 were also generated. Site-specific mutagenesis was performed by overlap extension PCR (Ho *et al.*, *supra*). Bacterial strains used as host for transformation were JM109 or MC1061.

For the construction of the chimeric h/r IgE molecule we employed the ε-chain expression plasmids PSV-VNphε/rε Helm *et al.*, *supra* (1991). A construct where the sequence known to be essential for hFcεR1 interaction has been replaced by the homologous rat sequence encoding residues 341-356 was also generated by overlap extension PCR. See Ho *et al.*, *supra*. The template for PCR was a 3.4 kb IgE $Cε_{1-4}$ genomic DNA cassette cloned into the BamHI site in pUC19 (pHε). A 719 bp fragment coding essentially for Cε2-3 was generated by PCR.

This involved PCR reactions employing four primers, two external primers 5'- CGTGAAGATCTTACAGTCGTC -3' (Seq. I.D. No. 6)and 5'- CCTGCCCATGGCTCACCG -3' (Seq. I.D. No. 7), and two internal primers 5'- CCTCGACCTGTATGAAAATGGG ACTCCCAAACTTACCTGTCTGGTGGTGGACCTG -3' (Seq. I.D. No. 8) and 5'-CCATTTTCATACAGGTCGAGGGGACTGGGTGGGATTAGGTAGGCG-CTCACCCCTCT -3' (Seq. I.D. No. 9). The external primers had *Bgl* II and *Nco* I restriction enzyme sites build in the sequence, respectively. For each PCR reaction, mixtures contained 200 ng of template, 2μg of each primer, 1 mM dNTPs, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$ in 100μl,

and following a hot start 1U Taq polymerase was added. An initial denaturation cycle at 96°C/6 mins, 64°C/2 mins, 72°C/1 min and 30 seconds was followed by 30 cycles 94°C/1 min 30 seconds, 64°C/1 min 30 seconds, 72°C/1 min 30 seconds. The resultant 719 base pairs fragment was cloned into pHe using the *Bgl II* and *Nco I* sites to give a chimeric Ce1-4 cassette which was subcloned, using the *BamHI* sites, into the mammalian expression vector pSV VNP (Helm *et al.*, *supra* (1991). The orientation of this cassette was checked by PCR.

The identity of all gene constructs was confirmed by sequencing the DNA of both strands.

Example 2. *Gene expression and purification of peptides*

E. coli strains transformed with the expression plasmids were grown overnight at 37°C and the overnight culture was diluted 100 fold into LB broth containing 100μg/ml ampicillin and grown to an absorbance of 0.4 at 600 nm at 37°C. The inducer IPTG (Sigma) was added to a final concentration of 0.1 mM and the cultures were grown under constant shaking at 37°C for 4h. Bacterial cells were harvested by centrifugation at 5,000g for 15 min and the pellets were frozen at -70°C until purification of the recombinant proteins. Freezing and subsequent thawing of the bacterial pellets were essential to obtain effective solubilization of the recombinant proteins which are expressed as insoluble inclusion bodies.

The chimeric PSV VNP construct was linearized using *Pvu I* and electroporated into the J558L plasmacytoma cell line. See Helm *et al.*, *supra* (1991). J558L cells were cultured in DMEM (10% FCS, Pen/Strep, Gentamicin) and selection medium (DMEM 10%FCS, Pen/Strep, Gentamicin, Mycophenolic acid, Xanthine and Hypoxanthine) was added 48 hours after electroporation. See Helm, *et al.*, *supra* (1991). High secreting clones were selected by ELISA.

Purification of recombinant GST-ε-chain fusion proteins from E. coli cell pellets - This was carried out using procedures described for the purification of recombinant ε-chain fragments expressed in *E. coli* (Helm *et al.*, *supra* (1988). Frozen cell pellets were defrosted on ice before homogenization (5 fold pellet volume) in 0.05 M Tris-HCl buffer pH 7.9 containing 2 mM EDTA, 0.1 mM dithiothreitol, 1 mM

β-mercaptoethanol, 0.25 M NaCl, 0.1% sodium deoxycholate, 25μg/ml PMSF, 5% glycerol. The homogenate were dispersed by sonication before addition of 100μg/ml lysozyme and 20μg/ml DNAase I. Homogenate were kept on a rotary shaker for 12-15h at 4°C before centrifugation at 10,000g for 10 min. The pellets were washed twice in 20 fold pellet volume of 0.05 M Tris-HCl buffer pH 7.9 containing 1 mM EDTA, 0.1 M NaCl, 25μg/ml PMSF. Inclusion bodies from cell pellets were solubilized in 0.05 M Tris-HCl buffer pH 7.9 containing 8 M urea, 1 mM EDTA, 0.1 M NaCl, 25μg/ml PMSF and dialyzed for 12h against 200 fold volume of the same buffer omitting urea, but with the addition of 0.1 mM dithiothreitol and 1 mM P-mercaptoethanol. Insoluble materials were removed by centrifugation and 30-75% of recombinant F-chain peptides were found in the supernatant fraction. Affinity purification from this fraction was carried out using a rabbit anti-GST antiserum coupled to Sepharose 4B. The chimeric h/r antibody was purified from cell culture supernatants using NP-specific affinity columns and analyzed by polyacrylamide gel electrophoresis (PAGE) and immunoblotting. See Helm, *et al.*, *supra* (1988); Helm *et al.*, *supra* (1991).

Gel electrophoresis and immunoblotting - PAGE and electroblotting procedures have been described before (Helm, *et al.*, *supra* (1988); Helm *et al.*, *supra* (1991). Blots were developed with a polyclonal horseradish peroxidase conjugated anti-hIgE antibody (Dako).

Example 3. *Ligand binding studies and cell culture*

hIgE VNP (Helm *et al.*, *supra* (1991)) was iodinated as previously described and the conditions for ligand binding and cell culture have been published (Wilson *et al.*, *supra*). Affinity purified GST-ε-chain fusion peptides were iodinated at 0-4°C in 0.4 M phosphate pH 7.4/7.5 using 4.4 μCi of $Na^{125}I$ and 150-300μg of peptide in tubes coated with 40 pg IODO GEN (Pierce). Following a 15 min incubation period, the reactions were terminated by removing fluid from the coated tubes. Each preparation was fractionated on a 140 ml Sephacryl S-200 column (Pharmacia), preequilibrated with binding

buffer (PBS/0.2% BSA pH 7.4), which effects the separation of dimers and monomers. Following γ-counting of collected fractions, peak fractions were pooled, aliquoted and stored at -70°C. Specific activity ranged from 5.8 - 15.5 μCi/μg.

The conditions for maintenance of RBL-2H3 cell-lines transfected with the α-chain of hFcεR1 (RBL-2/2/C) have been described. See Wilson *et al.*, *supra*. RBL2/2/C clones were plated into 48 well plates at an initial plating density of 105 cells/well and incubated with 10-6M dexamethasone for 24h at 37°C. In preliminary experiments, RBL-2/2/C cells were incubated with increasing concentrations of 1251 labelled ligands (0.1-7.5 pg/ml) to determine the minimum saturation concentrations for Fcε-R1 binding. The proportion of molecules capable of binding to FcεR1 was 78-91% for ^{125}I-labelled h and h/r IgE VNP, while the bindable portion of the ^{125}I-labelled GST-ε226-547, GST-ε326-547, GST-ε340-547, and GST-ε-226-354 was 41%, 56%, 53%, and 27% respectively. The number of rFεε molecules bound per cell was calculated on the basis that GST-ε326-547 and GST-ε340547 are dimers, while GST-ε-226-354 is a monomer.

Non-specific binding was determined using a 50-100 fold molar excess of non-labelled hIgE, and the same amount of GST was used as a negative control. The binding of recombinant proteins to FcεR1α was determined indirectly, after correcting for non-specific binding (7-17%), by calculating the percentage inhibition of ^{125}I-IgE bound to cells. To measure the inhibition (IC_{50}) of ^{125}I-IgE binding to RBL-2/2/C or the 8866 lymphoblastoid cell line (Helm *et al.*, *supra* (1993) by native and chimeric h/rIgE VNP, and recombinant ε-chain fragments, cells were pre-incubated with a 25, 50, 100, and 200 fold molar excess of each of the unlabelled peptides in ^{125}μl binding buffer or, as control, binding buffer alone at 22°C for 1h, before the addition of 50μl binding buffer containing 2μg/ml ^{125}I-IgE. After 45 min, the cells were washed twice with 0.5 ml ice cold binding buffer and lysed with 0.5 ml lysis buffer (0.5 M NaOH/1% Triton X-100). Samples (0-25 ml) were removed and counted for 5 min on a LKB1277 γ-counter.

The kinetics of association between RBL-2/2/C cells and ^{125}I-labelled h and h/r chimera, GST-ε226-547, GST-ε-326-547, GST-ε-340-547 (2μg/ml, in binding buffer) and GST-ε226-354, (0.7μg/ml, in

binding buffer) were measured at 22°C during the first 300 seconds of incubation at pH 7.4. Rate constants were calculated on the basis that GST-F-226-547. GST-ε326-547 and GST-ε-340-547 are dimers, while GST-ε226-354 is a monomer. The forward rate constant (K^{+1}) was calculated as VO/CO X RO, where VO represents the initial rate of binding, and CO and RO represent the concentration of ligand and receptor number (-130,000 hα-chains per cell. See Wilson *et al.*, *supra*. To determine the dissociation rate constant (K^{+1}), cells were pre-incubated for 1h at 22°C with $^{125}\mu$l of the ^{125}l-labelled h, and r IgE, the h/r chimera, GST-ε-226-547, GST-ε326-547, GST-ε340-547 and GST-ε226-354/7 (ligand concentration as for the determination of K^{+1}). Cells were washed twice with 0.5 ml binding buffer before $^{125}\mu$l binding buffer containing a 50 fold molar excess of unlabelled hIgE or binding buffer was added. At TO and after 15, 30, 60, 120, and 180 min intervals, the cells were washed twice with 0.5 ml ice cold binding buffer, solubilized in 0.5 ml lysis buffer (0.5 M NaOH/1% Triton X-100), and 0.25 ml samples were assayed for cell bound ^{125}I.

The pH optimum for the binding of ^{125}l-labelled hIgE and the GST-ε-chain fragments to FcεR1 was determined by incubating RBL-2/2/C cells (Wilson *et al.*, *supra*) in 48 well plates 50 mM phosphate buffered saline containing 0.2% BSA (pH range 5.9-8.1) for 10 min at 37° C before adding 50μl of 2μg/ml 125l-h or rIgE or 0.7 μg/ml of the ^{125}I-GST-ε fragments. Cells were incubated for 30 min and the excess-protein was removed by washing with saline containing 0.2% BSA before measurement of cell bound label. Results were corrected for nonspecific binding.

Potassium iodide titration - The generation of IgE mutant VNpheCys 328 -> Met has been described. See Helm *et al.*, *supra* (1991). The conformations of native and mutant (Cys 328 -> Met) recombinant IgE were investigated by comparing their intrinsic fluorescence. Solute quenching of protein fluorescence involved excitation of Trp residues at 297 nm and measurement of emission in the range 300-450 nm. Potassium iodide was added gradually to give a quench profile for each protein. Mathematical analysis was carried out according to the Stern-Volmer Law. See Lehrer, *Methods Enzymol.* **49**: 222-226 (1978).

Example 4. *Identification of core sequence*

The present study focused on the identification of the site(s) that determine the interaction of hIgE with its cellular receptors. The strategies employed for the expression of an overlapping family of chimeric GST-he-chain fusion proteins are outlined in Figure 1. Panels 1A & 1B summarize the receptor binding capacities of the GST-he-chain fusion proteins, that of a chimeric C/7 peptide, and a chimeric h/r IgE molecule. The assignation of biological activities is based on inhibition and direct binding studies detailed in Table 1. In Figure 1, panels C & D show the electrophoretic mobilities of C- and N-terminally truncated recombinant GST-he-chain fusion proteins immunoprecipitated with a rabbit anti-GST anti-serum, followed by PAGE analysis under non-reducing conditions, and immunoblotting with a horseradish peroxidase labelled rabbit anti-IgE serum. As shown in Figure 1C, C-terminal truncation yields a number of F--chain peptides for each construct. As judged by PAGE (Figure 1, panel C) and column chromatography (data not shown), approximately one third of the peptides in each set corresponds to the full length fusion peptide as a monomeric fragment. None of these fragments show any propensity to dimerize, although biologically inactive polymeric aggregates form at, concentrations >1.3 mg/mi. A set of identical fragments is observed following analysis under reducing conditions (data not shown). Most of the smaller e-chain fragments represent proteolytic cleavage fragments which are recognized by monoclonal antibodies specific for the Cε2 domain (data not shown). In contrast, deletion of N-terminal sequences gives rise to two ε-chain fragments and their apparent molecular weight under nonreducing (Figure 1, panel D) and reducing conditions (data not shown) indicates that they correspond, in almost equal quantities, to monomeric and dimeric GST-he-chain fusion proteins.

The data show that only those polypeptides that contain Cε4 or the homologous Cγ3 domain can engage both FcεR1 and FcεR2 while C-terminal truncation of the ε-chain results in elimination of binding to Fcε-R2. See Helm *et al.*, *supra* (1988); Vercelli *et al.*, *supra*. As shown in Figure 1 panel 1A, sequences common to all fragments

capable of binding to hFcεR1α comprise residues Pro343-Ser353 in the Cε3 domain. Further deletion from either the C- or N-terminal end beyond these residues is associated with a loss of FcεR1 binding.

As shown in Figure 2 and Table I, GST·ε-(340-547) and GST·ε-(226-354), which comprise the core peptide, inhibit the binding of hIgE with an IC_{50} in the nanomolar range. In contrast, blocking of IgE/FcεRI interaction by the GST controls GST·ε-(226-340), GST·ε-(355-547), and GST·ε-(355-547) cannot be detected even above micromolar concentrations.

As shown in the model structure of hIgE-Fc of Figure 3, this sequence forms a loop that is homologous to the loop in rIgG shown to bind to the neonatal FcγRn. See Burmeister *et al.*, *Nature* **372**: 379-384 (1994). A further similarity emerged when we investigated the pH dependency of the binding of hIgE to FcεR1. As shown in Figure 3 two pH optima are observed for the binding of hIgE to FcεR1, and occupancy of the receptor is almost twice as high at pH 6.4 than at 7.4. Although the significance of this is not known, it is tempting to speculate that IgE has evolved the lower pH optimum as a result of its physiological importance in the fight against parasitic infestations in the lumen of the intestine at acid pH.

Data summarized in Table 1 show that N- or C-terminal truncation has a negligible effect on the rate of association of biologically active ε-chain fragments with FcER1. In contrast, the rate of dissociation increases several hundred fold following the deletion of residues from the C-terminal end and, as shown in Figure 3, this is associated with a concomitant decrease in receptor occupancy at pH 6.4. Taken together, these data suggest that His residues in the C-terminal region of the IgE molecule make a contribution towards the maintenance of the high affinity interaction between IgE and FcεR1α since this is largely determined by the slow rate of dissociation of the ligand from the receptor.

The data show that the essential determinant for hIgE/FCεR1 recognition depends on a consecutive sequence comprising eleven amino acids computed to form a loop at the interface between the Cε-3 and Cε-4 domain. These observations exclude any direct contribution of Cε4-specific residues as proposed by Stanworth *et al.* See Stanworth

et al., *supra.*; Weetall *et al.*, *J. Immunol.* **145**: 3849-54 (1990); and Nissim *et al.*, *supra* (1991, 1992, and 1993). These results confirm and extend those made by Nissim *et al.*, who demonstrated that the receptor binding site in IgE is located in the Cε3 domain, and differ from the conclusions of Hamburger and Gould *et al.* Thus, Gould *et al.*, propose that residues 330-334 and 329-340 in the switch region between Cε2 and Cε3 are essential for IgE/FcεRI binding. See Hamburger, *Science* **189**: 389-391 (1975) and Gould *et al.*, *Biochem. Soc. Trans.* **21**: 968-972 (1993); *Nature* **366**, 421-428 (1993). As the results of our study clearly demonstrate, these sequences can be deleted without any major influence on the kinetics of hIgE/FcεRI interaction.

It is interesting to note that the active core sequence identified by us overlaps the hIgE-derived peptide generated by Nio *et al.*, who report its capability to block the binding of antigen-specific IgE to cells expressing FcεRI at concentrations in the mM range. See Nio *et al.*, *Peptide Chemistry* **2**, 203-206 (1989).

The results of our study indicate that fragments containing the Cε-2 domain are susceptible to proteolysis (Figure 1, panel C). The inclusion of the protease inhibitor phenyl methyl sulfonyl fluoride (PMSF) during the isolation procedure facilitates the purification of polypeptides that engage FcεRl and FcεR2.

Sequences N- or C-terminal to this core peptide are necessary to provide structural scaffolding for the maintenance of a receptor binding conformation since the core peptide alone cannot engage the receptor. Deletion of C-terminal, but not N-terminal sequences diminishes receptor occupancy at pH 6.4 and increases the dissociation of the ligand from the receptor, and we conclude that residues, including His, in the C-terminal domain make an important contribution towards the maintenance of the high-affinity of interaction between IgE and FcεR1α. Thus, the sequence herein identified as a core sequence overlaps but is different from those previously identified by others.

TABLE I

Binding of native and recombinant human and rat IgE-derived ε-chain fragments to hFcεRIα

The kinetics of association and dissociation of the affinity-purified ligands were determined at 22°C. Kinetic constants and IC_{50}, which represents the concentration at which 50% inhibition of ^{125}I-IgE $V_{NP}h\varepsilon$ binding was obtained, were calculated on the basis that GST · ε-(226-547), GST · ε-(326-354), and GST · ε-(340-547) form dimers, while GST · ε-(226-354) is a monomer. Nonbinding ligands used at 10^{-5} M showed no binding above background. Data shown represent the calculated mean of experiments carried out in duplicate.

Ligand	k_{+1} $M^{-1}s^{-1}$	k_{-1} s^{-1}	K_d M	IC_{50} nM	n
hIgE	3.14×10^5	1.3×10^{-5}	4.1×10^{-11}	14	>8
hIgE rε-(341-356)	4.3×10^4	1.7×10^{-5}	3.9×10^{-10}	ND[a]	1
GST ε-(226-547)	3.6×10^5	1.6×10^{-5}	4.4×10^{-11}	18	2
GST ε-(326-547)	4.1×10^5	2.95×10^{-5}	7.2×10^{-11}	23	2
GST ε-(340-547)	4.2×10^5	9.8×10^{-5}	2.3×10^{-10}	34	2
GST ε-(226-354)	4.3×10^5	6.02×10^{-3}	1.4×10^{-8}	332	3

[a] not determined

Example 5. *Anti-oligopeptide polyclonal serum production*

Anti-sera from rabbits were obtained by immunization with both constrained and unconstrained oligopeptide. The oligopeptide employed was C L S R P S P F D L F I R K S P T I T S C C. The constrained form was obtained by incubation of 0.5 mg/ml of oligopeptide dissolved in 50 mM ammonium bicarbonate for two hours with stirring at room temperature, and then for 90 hrs. at 4°C. The formation of the constraining disulfide bond and the formation of the oligopeptide loop were followed by mass spectrometry; loop formation occurred after three days of standing. After 90 hrs. the sample was lyophilized. Two rabbits each were used. Rabbits were immunized by subcutaneous injection with 100 micrograms each of oligopeptide suspended in PBS/FCA (PBS = phosphate buffered saline, FCA = Freund's complete adjuvant) (v/v). At two-week intervals, the rabbits

received another dose of immunogen in the same concentration but this time suspended in PBS/FIA (FIA = Freund's incomplete adjuvant) (v/v). After the third and last injection (in PBS/FIA), the serum was screened by ELISA for the presence of polyclonal antibodies against the oligopeptide immunogen. Four days after a final intravenous injection (boost), the blood was collected and left at room temperature until clotted. The serum was collected with a Pasteur pipette followed by centrifugation at 3,000 rpm for 10 min in order to separate remaining erythrocytes. Serum aliquots of 1 ml were transferred to Eppendorf tubes and frozen at -20° C until further use. The titer for the constrained oligopeptide used in ELISA was 1:1000; that for the unconstrained oligopeptide it was 1:100.

Example 6. *Inhibition of IgE binding to receptor*

Three different parameters were investigated.

(1) RBL-2/2C cells were incubated overnight with 5-hydroxytryptamine (5-HT) and with JW8 IgE (Serotec USA, Wash. D.C.) for 24 hours. See Wilson *et al., supra* (1993a, 1993b). The cells were washed 2 times and challenged with the anti-sera raised against the oligopeptides diluted 1:50, 1:100, 1:250, 1:500, and 1:1000. After 15 min, 5-HT release was determined. No 5-HT release was observed under these conditions. In control experiments, cells were incubated overnight in the same fashion, but challenged on day 2 with either JW8 IgE-specific antigen, *i.e.* 5-iodo,4hydroxy,3-nitrophenyl linked to human serum albumin (NIP-HSA) or a monoclonal antibody directed against the Cε2 or Cε4 domain, approximately 30% of total cellular 5-HT content was released. This shows that the anti-constrained oligopeptide antiserum does not trigger release of 5-HT by cells sensitized with IgE.

(2) RBL-2/2/C cells were incubated with 5-HT for 24 hrs. after which the cells were washed 2 times as above. 0.5 ug/ml of JW8 IgE was incubated overnight with anti-constrained and unconstrained oligopeptide serum (diluted in buffer 1:5, 1:10, 1:50, 1:100, 1:250). 5-HT release was measured after a 15-min incubation period. No 5-HT release was observed. In control experiments where IgE was incubated overnight in the same fashion with monoclonal antibodies directed

against epitopes on either the Cε2 or Cε4 domain, approximately 30% of total cellular 5-HT content was released when these were added to the cells.

(3) RBL-2/2/C cells were incubated with 5-HT for 24 hours after which the cells were washed 2 times as before. 0.5 ug/ml of JW8 IgE was incubated overnight with anti-serum against the constrained and unconstrained oligopeptides (diluted in buffer 1:5, 1:10, 1:50, 1:100, 1:250). The IgE and anti-serum were added to the washed cells and these were incubated for 1 hour in a tissue culture incubator. For conditions, see Wilson *et al.*, *supra* (1993). Cells were washed 2 times with buffer, NIP-HSA was added (10 ng/ml), and 5-HT release measured after 15-min of incubation. No 5-HT release was observed when cells were incubated with the anti-serum against the constrained oligopeptide at dilutions between 1:10 to 1:100. In control experiments, cells sensitized with 0.5 ug/ml NIP-specific JW8 IgE, followed by challenge with NIP-HSA, released approximately 30% 5-HT. Cells that were incubated with the anti-serum against the unconstrained oligopeptide, where this was diluted 1:10, showed a nearly 30% release of 5-HT.

The formation of the disulfide-constrained oligopeptide was monitored using a V9 platform (Fisons) Electron Spin Resonance spectroscopy with positive electrospray vaporization, where the mobile phase had 50% acetonitrile, 50% water plus 1% formic acid added to the sample mix with approximately 1 ug of oligopeptide. Disulfide bond formation occurred after 3 days standing.

Although the foregoing refers to particular preferred embodiments, it will be understood that the present invention is not so limited. It will occur to those of ordinary skill in the art that various modifications may be made to the disclosed embodiments and that such modifications are intended to be within the scope of the present invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Padlan et al.

(ii) TITLE OF INVENTION: Inhibition of IgE-Mediated Allergies By A Human IgE-Derived Oligopeptide

(iii) NUMBER OF SEQUENCES: 9

(iv) CORRESPONDENCE:

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(E) COUNTRY: U.S.A.

(F) ZIP: 97204-2988

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5" Disk

(B) COMPUTER: IBM-PC compatible

(C) OPERATING SYSTEM: Windows NT

(D) SOFTWARE: WordPerfect 7.0/ASCII

(vi) CURRENT APPLICATION DATA: Filed herewith

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

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(A) APPLICATION NUMBER: U.S. Provisional 60/031,991

(B) FILING DATE: December 12, 1996

(viii) ATTORNEY/AGENT INFORMATION

(A) NAME: David J. Earp, Ph.D.

(B) REGISTRATION NUMBER: 41,401

(C) REFERENCE/DOCKET NUMBER: 4239-48989

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (503) 226-7391

(B) TELEFAX: (503) 228-9446

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) SEQUENCE DESCRIPTION: SEQ ID NO.:1:

Pro Ser Pro Phe Asp Leu Phe Ile Arg Lys Ser
5 10

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) SEQUENCE DESCRIPTION: SEQ ID NO.:2:

Cys Leu Ser Arg Pro Ser Pro Phe Asp Leu Phe Ile Arg Lys Ser
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Pro Thr Ile Thr Ser Cys Cys
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) SEQUENCE DESCRIPTION: SEQ ID NO.:3:

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5 10 15

Thr Ile Thr Cys Cys
20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) SEQUENCE DESCRIPTION: SEQ ID NO.:4:

Cys Arg Pro Ser Pro Phe Asp Leu Phe Ile Arg Lys Ser Pro Thr
5 10 15

Ser Cys Cys
18

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) SEQUENCE DESCRIPTION: SEQ ID NO.:5:

Cys Pro Ser Pro Phe Asp Leu Phe Ile Arg Lys Ser Pro Thr Cys
5 10 15

Cys
16

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) SEQUENCE DESCRIPTION: SEQ ID NO.:6:

CGTGAAGATC TTACAGTCGT C 21

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) SEQUENCE DESCRIPTION: SEQ ID NO.:7:

CCTGCCCATG GCTCACCG 18

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 55

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) SEQUENCE DESCRIPTION: SEQ ID NO.:8:

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(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 56

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) SEQUENCE DESCRIPTION: SEQ ID NO.:9:

CCATTTTCAT ACAGGTCGAG GGGACTGGGT GGGATTAGGT AGGCGCTCAC CCCTCT 56

What Is Claimed Is:

1. A purified oligopeptide that interacts with the Fcε receptor, wherein the oligopeptide comprises the amino acid sequence:

P S P F D L F I R K S.

2. The purified oligopeptide according to claim 1 wherein the oligopeptide is fewer than 50 amino acids in length.

3. The purified oligopeptide according to claim 2 wherein the oligopeptide is 13-27 amino acids in length.

4. The purified oligopeptide according to claim 1, wherein said oligopeptide has been constrained to form a loop structure.

5. A purified oligopeptide comprising an amino acid sequence selected from the group consisting of the sequences set forth in Seq. I.D. Nos. 1-5.

6. A pharmaceutical preparation comprising a purified oligopeptide according to claims 1, 2, 3, 4 or 5.

7. An isolated DNA molecule encoding an oligopeptide according to claims 1, 2, 3, 4 or 5.

8. A biological system that expresses the DNA molecule of claim 7.

9. A biological system according to claim 8, wherein said biological system is a prokaryotic or a eukaryotic cell.

10. A method of preparing an oligopeptide competitor of IgE, comprising:

(a) culturing a prokaryotic or eukaryotic cell according to claim 9, and

(b) purifying said oligopeptide.

11. A competitive binding assay for hIgE wherein the competitor is an oligopeptide according to claims 1, 2, 3, 4 or 5.

12. An antibody raised against a purified oligopeptide according to claims 1, 2, 3, 4 or 5.

13. A pharmaceutical preparation comprising an antibody according to claim 12.

14. A diagnostic method for determining IgE levels in a sample of fluids comprising the steps of:

(a) contacting said sample with an antibody raised against an oligopeptide competitor to IgE; and

(b) precipitating IgE with said antibody.

15. The method of claim 14 wherein the antibody is an antibody raised against a purified oligopeptide according to claims 1, 2, 3, 4, or 5.

16. A diagnostic method of determining available Fce receptors on the membrane of cells, comprising the steps:

(a) providing a sample of said cells;

(b) providing a labeled oligopeptide competitor to IgE; and

(c) determining the amount of label bound by said cells.

17. A kit for a diagnostic method for determining IgE levels, comprising an antibody of claim 12 and a container for said antibody.

18. A diagnostic kit for determining available Fce receptors comprising a purified oligopeptide according to claims 1, 2, 3, 4 or 5, and a container for said polypeptide.

19. A method of targeting a cell which expresses FceR1 receptor, comprising delivering into an organism an oligopeptide according to claim 1.

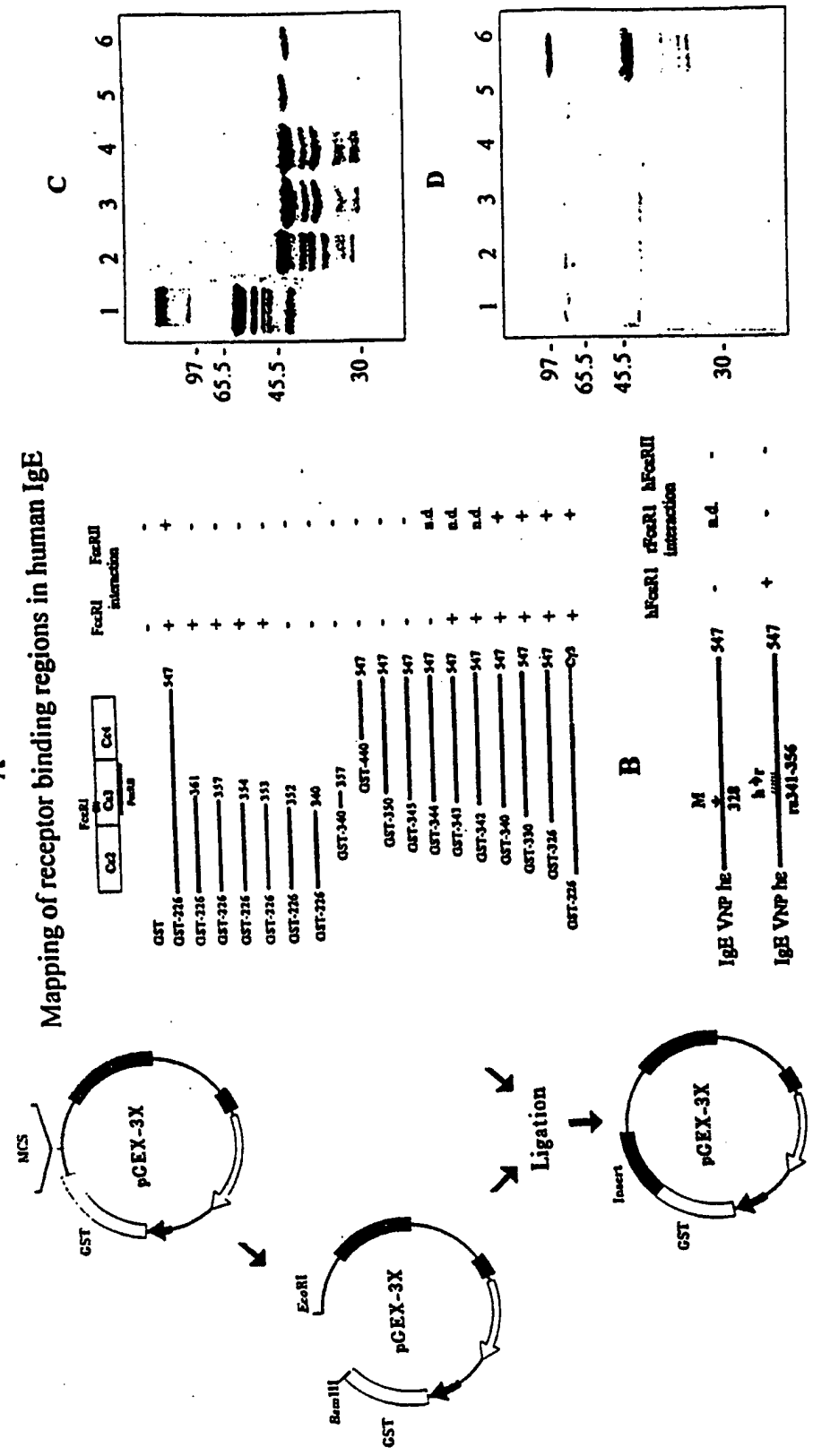
20. A protein that interacts with the FceR1 receptor, wherein the protein consists essentially of the amino acid sequence P S P F D L F I R K S.

21. A protein that competes with IgE for the FceR1 receptor, wherein the protein consists essentially of the amino acid sequence selected from the group consisting of the sequences set forth in Seq. I.D. Nos. 1-5.

22. A protein that competes with IgE for the FceR1 receptor, wherein the protein consists essentially of:

(i) an amino acid sequence P S P F D L F I R K S; and

(ii) constraining residues that flank the amino acid sequence of (i), wherein the constraining residues include cysteine and are within 16 residues N-terminal and 4 residues C-terminal of the amino acid sequence of (i).


A
Mapping of receptor binding regions in human IgE
FcεRI
Cε2
Cε3
Cε4
FcεRII
FcεRI interaction
FcεRII interaction
GST
GST-226
GST-225
GST-336
GST-334
GST-333
GST-332
GST-340
GST-337
GST-440
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GST-345
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GST-343
GST-342
GST-340
GST-330
GST-326
GST-225
Cε3
n.d.
B
hFcεRI
rFcεRI
hFcεRII interaction
M
IgE VNP he
318
IgE VNP he
m341-356
C
D
97-
65.5-
45.5-
30-
pGEX-3X
MCS
GST
EcoRI
BamHI
Ligation
Insert

FIGURE 1

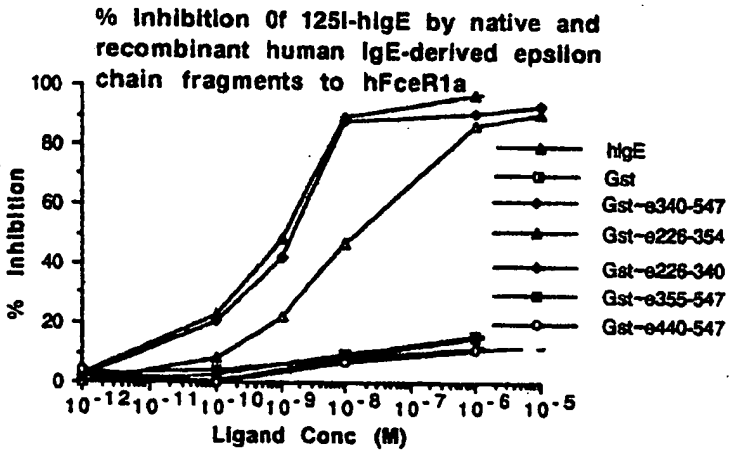
% Inhibition Of 125I-hIgE by native and recombinant human IgE-derived epsilon chain fragments to hFceR1a
% Inhibition
100
80
60
40
20
0
10-12 10-11 10-10 10-9 10-8 10-7 10-6 10-5
Ligand Conc (M)
hIgE
Gst
Gst-e340-547
Gst-e228-354
Gst-e228-340
Gst-e355-547
Gst-e440-547

FIGURE 2

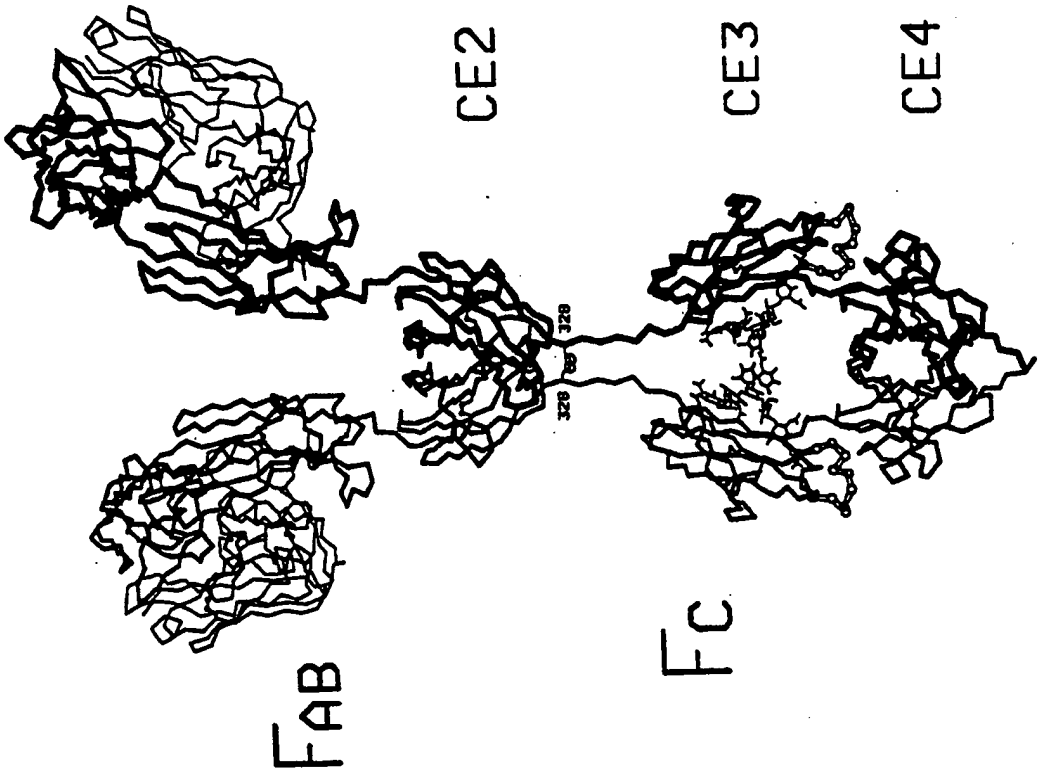

FIGURE 3

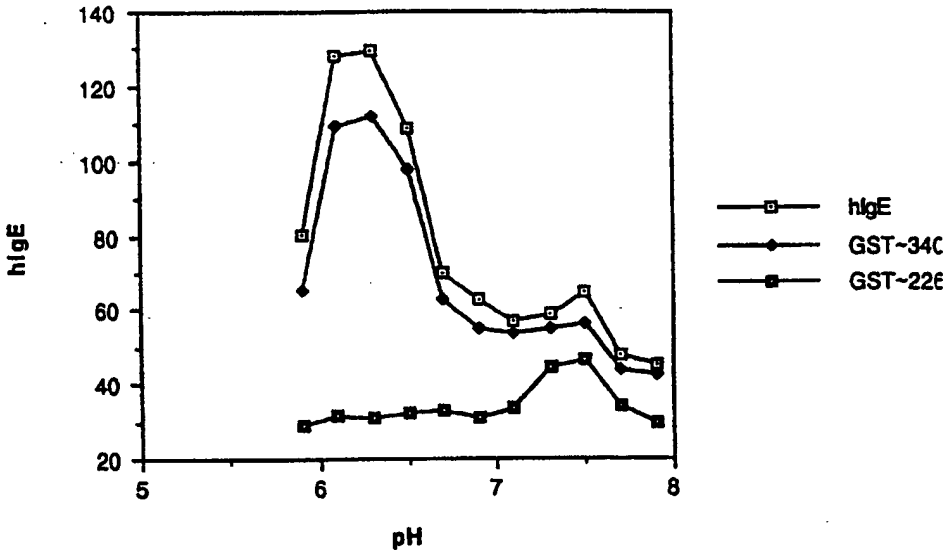

FIGURE 4